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A GENETIC AND MORPHOLOGICAL STUDY OF *CLARKIA STELLATA* (ONAGRACEAE) AND RELATED SPECIES IN NORTHEASTERN CALIFORNIA

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Abstract

Clarkia stellata Mosquin (Onagraceae) is an uncommon annual herb endemic to Plumas and Yuba Counties in northeastern California that has threatened populations due to noxious weeds, recreational and forest management activities, and development. The purpose of this study is clarify the species identification of populations of *Clarkia stellata* for management purposes, specifically, populations of *C. stellata* and *C. rhomboidea* that are difficult to differentiate in the field. A total of 11 populations of *C. stellata* and related species were sampled for morphometric analyses and nine populations were sampled for genetic analysis using amplified fragment length polymorphisms (AFLPs). *Clarkia stellata* can be separated from *C. rhomboidea* based on all floral characteristics except claw width, claw length, and isthmus width. These species also can be differentiated based on the following vegetative characteristics: petiole length, leaf length, leaf width, and plant height. The sympatric *Clarkia mildrediae* is easily differentiated from *C. stellata* by every character except petiole length, leaf length, and plant height; *C. mildrediae* differs from *C. rhomboidea* for all characters except petal speckling, pollen color, leaf width, and leaf length. Populations that were initially difficult to categorize as either *C. stellata* or *C. rhomboidea* were most similar to *C. stellata*; however, we were not able to identify a suite of characters that would distinguish these populations as either *C. stellata* or *C. rhomboidea.* An analysis of molecular variance (AMOVA) shows that although there was genetic variation among all populations (13.19%), the majority of variation is found within populations (86.81%). Genetic differentiation among all populations was low as calculated by Genetic Data Analysis (Φ_{st} = 0.132) and Hickory (θ^B = 0.0137); variance within populations was high (sigma-G = 32.645) and between populations was low (sigma-P = 4.96).

This work is consistent with a number of studies within *Clarkia* section *Myxocarpa* that have identified taxonomic difficulties due to recent speciation, local adaptation, rapid chromosomal evolution, sympatry, and hybridization.

Key Words: *Clarkia,* Amplified Fragment Length Polymorphisms, rare species, morphometrics, rapid evolution.

Clarkia stellata Mosquin (Onagraceae) was described from Lake Almanor, Plumas County and nearby Yuba County in Northeastern California (Mosquin 1962); it is now known to occur uncommonly in coniferous forest openings at elevations from 1000 to 1500 m within Plumas, Tehama, Nevada, Placer, and Yuba counties (Lewis 1993).

Threats to populations of *C. stellata* include noxious weeds, timber harvest activities, reforestation, livestock grazing, lack of fire, fire fighting/suppression activities, spring prescribed burning, camping, mining, road construction and maintenance, and development (Van Zuuk 2000). *Clarkia stellata* is not listed as rare, endangered, or threatened by the state or federal government; however, because of its uncommon occurrence, it is considered a "sensitive species" by the U.S. Forest Service (Van Zuuk 2000).

Clarkia stellata is included within *Clarkia* section *Myxocarpa* which includes the diploid species *C. australis* Small, *C. borealis* Small, *C. mildrediae* (Heller) Lewis and Lewis, *C. mosquinii* Small, *C. virgata* Greene, and the polyploid *C. rhomboidea* Douglas (Small 1971a). *Clarkia stellata* and *C. rhomboidea* are presumed to be autogamous based on simultaneous maturation of the stigma and anther. Extensive hybridization and chromosomal analyses strongly support that *C. stellata* originated as a result of one or more reciprocal translocations in *C. mildrediae* (Mosquin 1961; Small 1971a, b).

Although *Clarkia stellata* is morphologically most similar to *C. rhomboidea,* based on chromosomal evidence, *C. rhomboidea* is hypothesized to have formed from hybridization between *C. virgata* and *C. mildrediae* (Mosquin 1964). *Clarkia rhomboidea* is common in yellow pine forests and woodlands at elevations less than 2500 m throughout the California Floristic Province and beyond. Although Small (1971) noted that *C. stellata* tends to occur on more xeric microsites than *C. rhomboidea,* these species often occur sympatically and are indistinguishable in the vegetative state. Sympatric is defined here as occurring in close proximity but not in mixed populations, with the exception of the "*C. stellata/rhomboidea*" populations, which are mixed.

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TABLE 1. MORPHOLOGICAL CHARACTERS FOR *CLARKIA STELLATA, C. RHOMBOIDEA, C. MILDREDIAE,* AND *C. MOSQUINII* AS PROVIDED BY LEWIS (1993). Abbreviations: lbd = lobed; incons. = inconspicuously.

Species	Petal length (mm)	Petal spotting	Claw/Blade	Stigma > anthers?	Chromosome #	Pollen color
C. stellata	6–8	no	claw 2-lbd/ incons. 3-lbd	no	$n = 7$	yellow
C. rhomboidea	7–14	generally spotted	claws 2-lbd/ unlobed	no	$n = 12$	blue-gray
C. mildrediae	15–20	flecked/ spotted	claw 2-lbd/ unlobed	yes	$n = 7$	light blue/ blue
C. mosquinii	15–20	purple spotted	claw 2-lbd/ unlobed	yes	$n = 6$	blue-gray

Species within *Clarkia* section *Myxocarpa* are notoriously difficult to distinguish in the field (Small 1971; Gottlieb and Janeway 1995). Although some morphological characters superficially separate the Section *Myxocarpa* species that occur sympatrically in northeastern California (*C. stellata, C. rhomboidea, C. mildrediae, and C. mosquinii* [Table 1; Lewis 1993]) overlapping variation has been identified frequently in the field (L. Janeway personal observation).

The purpose of this study is not to provide a definitive study of members of *Clarkia* section *Myxocarpa* but is to clarify the identification of populations of *C. stellata* for management purposes. Specifically, populations of *C. stellata* and *C. rhomboidea* in northeastern California are often difficult to differentiate, and our null hypothesis is that populations of these species cannot be separated morphologically and genetically.

MATERIALS AND METHODS

Morphometric Data Collection

Two populations ambigously identified as *Clarkia "stellata/rhomboidea"*, known populations of *C. stellata* (six) and *C. rhomboidea* (two), and one population of *C. mildrediae* (included because of its sympatry with a *C. "stellata/rhomboidea"* population) were sampled in June and July 2001 (Table 2). In the *"C. stellata/rhomboidea"* populations, individuals were selected for analyses based on their ambiguous morphological characters. *Clarkia mosquinii* was not included in this study because of its rarity, it is not easily confused with the other study species, and it has not been reported growing with *C. stellata.* Populations were sampled based on records provided by the U.S. Forest Service and based on an *a priori* determination that used the characters provided by Lewis (1993). Some difficulty was encountered in finding populations with enough flowering plants for adequate sampling due to low rainfall during the winter of 2000–2001. Material was collected from a total of 210 individuals, where n = 16–20 per population. Voucher specimens for all populations are on file at the CSU Chico Herbarium (CHSC).

We followed the protocol of Gottlieb and Ford (1999) for petal measurements and collected only from plants that had open stigmas. The following floral characteristics were measured: limb width, "isthmus" width at the narrowest point, claw width, and claw length. Petal speckling was assigned a value of 1 (none), 2 (slight), 3 (sparse), 4 (moderate), and 5 (dense). Pollen color was assessed on fresh material in the field and assigned a value of 1 (yellow and light yellow), 3 (light green), and 5 (light blue and blue). The vegetative characters, leaf length, leaf width, petiole length, and plant height were also measured.

Morphometric Data Analysis

Descriptive statistics were calculated for each population for all measured characteristics. Differences among "species" categories were analyzed using a Kruskal-Wallis One Way Analysis of Variance on Ranks. All pairwise multiple comparison procedures were calculated using Dunn's method. Petal speckling is often cited as a key character, thus we performed a multiple linear regression analysis between this and all other morphological characteristics to estimate its reliability in identifying the study species. All morphological statistics were calculated using SigmaStat 3.2 (SPSS, Inc. CA USA).

Genetic Data Collection

Leaf samples were put on ice and directly transported to California State University, Chico (CSU Chico), where they were stored at −80°C until DNA extraction. Genomic DNA was extracted from the same individuals used in the morphometic analysis using a Fast Prep Kit (Bio 101, Inc); however, population sample sizes ranged from 6–20, and the HUMB and MDWV populations were not included, due to difficulties in the extraction and PCR process. DNA concentrations were determined using a GeneQuant (Pharmacia Biotech), and the samples stored at −20°C until needed.

AFLP digestion, ligation, and PCR-amplifications were carried out using an AFLP Analysis System (Vos et al. 1995; GibcoBRL, Life Technolo-

TABLE 2. COLLECTION LOCATIONS FOR *CLARKIA STELLATA*, *C. RHOMBOIDEA*, *C.* "*STELLATA/RHOMBOIDEA*", AND *C. MILDREATAE* POPULATIONS. PSME = *Pseudotsuga menziesii*, MCF = Mixed conifer forest, YPF = Yellow pine forest, ABCO = *Abies concolor*, PIJE = *Pinus jeffreyi*, PIPO = *Pinus ponderosa*, LIDE2 = *Lithocarpus densiflora*.

Population/ Coll. date	Forest type	Elev.	Lat./Long.	U.S.G.S. Topo. Map
Clarkia stellata				
CALF 6-15-01	PSME-MCF	1402 m	40°09′39″, 121°31′43″	Onion Butte, se ¼ of sw ¼ sect. 23, T27N, R4E
Comments: Population numbers in 1000's (*Janeway 7174* CHSC). Mid to late flowering, ca. 25% w/fruits only. Slope 40°, aspect SW. Above N. Fork of Calf Creek, 1.6 km N–NW of Colby Mtn. and between 27N12 and 27N06. A clearcut unit, shrubs dominate, especially *Ceanothus intergerrimus*, 3.5 m tall. Pines planted 5–6 years old, 1 m fall. Burned following harvest. Stigma receptive, stigmas shorter than stamens, pollen "light green"				
HUMB 6-15-01	YPF-ABCO	1384 m	40°12′38″, 121°12′27″	Almanor, nw & sw ¼ of sw ¼ sect. 9, T27N, R7E
Comments: Population approx. 400 plants (*Janeway 7179* CHSC). Slope ~0°, aspect southerly. E. side of Humbug Rd ca. 2.72 km SW of shore of Lake Almanor, 4 km W of Prattville. Rocky volcanic, *Clakria stellata* esp. where rock outcrops to surface and fractures (less logging and disturbance). Stigma receptive w/pollen. Anthers dehisced, anther sacs and pollen yellow.				
MDWV 6-14-01	PSME-MCF	1329 m	39°54′54″, 121°00′20″	Meadow Vly, sw ¼ of nw ¼ sect. 29, T27N, R9E
Comments: Population approx. 500 plants (*Janeway 7173* CHSC). Approx. 75% in fruit only. Slope 10–35°, aspect S, SW. Deep soil, lightly rocky, sandstone/shale substrate. "Plantation" recently thinned, probably brushed, partly burned including tractor trails. Stigmas receptive w/pollen.				
CONE 6-28-01	YP-MCF	~1768 m	40°41′36″, 121°07′11″	Harvey Mountain, sect. 30 T33N, R8E
Comments: Population approx. 200 plants (*Janeway 7233* CHSC). Approx. 5% in bud, 45% in flower, and 50% in fruit. Slope 10°, aspect SW. SW flank of Cone Mtn, 0.48 km SW of the summit and along road. Fairly consistent speckling, pollen color, and anther sac color.				
MONT 6-24-01	PIJE-MCF	1676 m	40°16′54″, 121°39′28″	Lyonsville se ¼, of nw ¼ sect. 17, T28N, R3E
Comments: Population approx. 500 plants (*Janeway 7216* CHSC). Approx. 10% in bud, 60% in flower, and 30% in fruit. Slope 10–20°, aspect SW. On Monterey Point ridge 0.16 km SW of Rd 29N48; ca 4 km SW of Turner Mtn. SW-trending ridgetop. The red-orange anthers are often quite showy, esp. when not open all the way. Stigma receptive and coated with pollen.				
WILL 6-19-01	YPF-MCF	1658 m	40°24′20″, 121°21′36″	Mt. Harkness nw ¼ of nw ¼ sect. 6, T29N, R6E
Comments: Population approx. 400 plants (*Janeway 7185* CHSC). Approx. 30% in bud, 60% in flower, and 10% in fruit. Slope 30–40° aspect S, SW. Willow Lake at scattered locations along the north side of the lake, above trail 3–15 m. Rocky volcanic. Areas with *Clarkia stellata* fairly to quite exposed in afternoon.				
Clarkia rhomboidea				
JCTH 6-17-01	PSME-MCF-LIDE2	1008 m	39°43′40″, 121°18′06″	Brush Crk. sw ¼ of sw ¼ sect. 27, T22N, R6E
Comments: Population > 300 plants (*Janeway & Castro 7183* CHSC). Approx. 10–20% in current and recent flower and rest in fruit. Slope 40–50°, aspect SW. 1.6 km SW of Junction House along Rd22N49 parallel to and about 100 m of OroQuincy Hwy. revegetating PIPO plantation. Pollen blue, anther pacs magenta like filaments and style, sigmas receptive, longer than usual, just > anthers, bend down away from anthers. **Chromosome counts (Gottlieb & Janeway 1995) verify this population as *C. rhomboidea*.				
GANS 6-28-01	PSME-MCF	1069 m	40°02′12″, 121°13′39″	Caribou, nw ¼ of sw ¼ sect. 8, T25N, R7E
Comments: Population approx. 200 plants (*Janeway 7238* CHSC). Approx. 10% in bud, 30% in flower, and 50% in fruit. Slope 50°, aspect S, SE. On slope of ridge overlooking North Fork Feather River, and Caribou Road ca. 2.56 km N of confluence of E. Br. N. Fk. Feather River and along rd 3.36 km from Caribou Road.				

gies, 1996) with some modifications. Approximately 250 nm of genomic DNA were double digested with the restriction enzymes *Eco*R1 and *Mse*1. The DNA and enzymes were mixed with a reaction buffer (50mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate), and distilled water, placed in a thermocycler for 2 hr at 37°C and incubated period at 70°C for 15 min. The samples were then cooled, adapters and DNA ligase added, and the mixture incubated for 2 hr at 20°C.

The DNA was diluted with TE buffer to a concentration of 1:10 and used in the first of two PCR

TABLE 2. CONTINUED.

Population/ Coll. date	Forest type	Elev.	Lat./Long.	U.S.G.S. Topo. Map
Clarkia rhomboidea/Clarkia stellata				
WATS 7-3-01	PIPO-MCF	1707 m	38°50′22″ 121°02′45″	Dogwood Pk, ne ¼ of se ¼ sect. 23, T23N, R8E
Comments: Population approx. 200 plants (*Janeway 7258* CHSC). Approx. 10% in bud, 50% in flower, and 40% in fruit. Slope 20–35°, aspect S. Top of S-facing slope overlooking mid fork Feather River and head of Sherman Creek. A couple of *Clarkia mildrediae* ssp. *lutescens* in bud but the *Clarkia mildrediae* population is about 100 m east, flowering and without *C. stellata/C. rhomboidea.*				
HRIM 6-21-01	YPF-MCF	1524–1585 m	40°41′55″, 121°23′35″	Old Station, sect. 23 and 26 T33N, R5E
Comments: Population approx. 800 plants (*Janeway 7215* CHSC). Approx. 0% in bud, 10% in flower, 90%. Slope 20–35°, aspect NE. On top of Hat Creek Rim, 3.52 km NE of Old Station. Only about 25% as many plants as last year.				
Clarkia mildrediae				
WATM 7-3-01	PIJE-MCN	1707 m	39°50′23″, 121°02′40″	Dogwood Pk, nw ¼ of sw ¼ sect. 24, T23N, R8E
Comments: Population approx. 500 plants (*Janeway 7267* CHSC). Approx. 20% in bud, 80% in flower, and 0% in fruit. Slope 40°, aspect S–SW. Top of S–SW facing slope overlooking mid fork Feather River and head of Sherman Creek. Soil volcanic, somewhat rocky.				

programs. The samples were prepared for the first program by the addition of pre-amplification primer mix (0.94 μg/mL *Eco*R1 adapter, 0.94 μg/mL *Mse*1 adapter, 10mM dNTP's), 10X PCR buffer plus $MgCl_2$, and *Taq* polymerase. The PCR program was as follows: 94°C for 30 sec, 56°C for 60 sec, and 72°C for 60 sec for 20 cycles. The PCR product was diluted to a concentration of 100 ng of DNA.

The final PCR reaction was run with diluted DNA PCR product and two mixes. In mix 1, the *Eco*R1 and *Mse*1 primers were selected and mixed together. Ten primer combinations were screened and ultimately, two AFLP primer set combinations (*Eco*R1/*Mse*1) were used to identify polymorphisms within and among populations. In mix two, 10X PCR buffer, distilled water, and *Taq* polymerase were mixed. Mix 1 and 2 were then combined with the diluted DNA and underwent the following PCR conditions: 94°C for 1 min, 65°C for 1 min; and 72°C for 1 min, 30 sec for 23 cycles.

AFLP-PCR products were separated electrophoretically on a non-denaturing 6% polyacrylamide gel at 1000 volts for two hours. The resulting banding patterns were visualized using silver staining (Cho et al. 1996). The gels were dried overnight and photographed using APC (automatic processor compatible) film from Promega (catalog # Q441) and a light table. The light exposed photo paper was developed using an X-ray film-processing machine.

Genetic Data Analysis

Presence (1) or absence (0) data from the AFLP gels were collected for each individual via manual scoring. Only fragments that were unambiguous were included in the analysis. These data were used to calculate genetic similarities using Pairwise squared Euclidean distances (Excoffier et al. 1992) constructed with a Euclidean Matrix Macros in Microsoft Excel (2000). Genetic similarities among populations were analyzed by the AMOVA program (version 1.55; Excoffier et al. 1992), which allows calculation of variance components and significance levels on several hierarchical levels, including within and among populations (Schierenbeck et al. 1996; Schmidt and Jensen 2000). PAUP version 4.0b8 was used to generate a Neighbor-Joining phylogram with the Upholt option in order to show associations among populations (Swofford 1998). Within population statistics for expected heterozygosity (He) and polymorphic loci (P) were calculated using Genetic Data Analysis (Lewis and Zaykin 2002). Hickory version 1.0 (Holsinger et al. 2002; Holsinger and Lewis 2003) was used to calculate the population statistics, f and θ^B, analogous to the F- statistics (Wright 1969) F_{IS} and F_{ST}, respectively. We used the Hickory default values for burn-in (50,000), sampling (250,000), and thin (50). Hickory uses Bayesian methods and specifically here, the Deviance Information Criterion (DIC), which fits the f model to the data, and allows a determination of inbreeding within populations or genetic differentiation among populations.

RESULTS

Morphological Analysis

Descriptive statistics for morphological characteristics are provided for all populations (Table 3). An ANOVA by species categorization indicates there are significant differences between *C. stellata* and *C. rhomboidea* for all characters except claw

TABLE 3. MORPHOMETRIC ANALYSIS FOR *CLARKIA STELLATA*, *C. RHOMBOIDEA*, *C.* "*STELLATA/RHOMBOIDEA*", AND *C. MILDREATAE* POPULATIONS IN NORTHERN CALIFORNIA. See Table 2 for population and species abbreviations. All values are means and standard errors, and values are in mm with the exception of plant height (dm) and pollen color and petal speckling (see materials and methods).

Species	Population	Petal claw length	Total petal length	Petal claw width	Petal isthmus width	Petal limb width
CLST	CALF	1.65 (0.030)	7.66 (0.102)	2.12 (0.045)	1.53 (0.031)	4.22 (0.095)
CLST	MDWV	1.36 (0.044)	6.39 (0.188)	2.15 (0.062)	1.75 (0.266)	3.85 (0.113)
CLST	WILL	1.49 (0.028)	7.36 (0.120)	1.96 (0.032)	1.68 (0.028)	4.08 (0.109)
CLST	CONE	1.74 (0.107)	7.62 (0.235)	1.56 (0.068)	1.49 (0.048)	4.19 (0.167)
CLST	HUMB	1.25 (0.059)	6.58 (0.153)	1.74 (0.059)	1.62 (0.053)	3.91 (0.117)
CLST	MONT	1.58 (0.050)	7.29 (0.122)	2.16 (0.047)	1.68 (0.023)	4.68 (0.116)
CLRH	JCTH	2.63 (0.086)	10.37 (0.290)	2.28 (0.068)	1.90 (0.036)	5.65 (0.231)
CLRH	GANS	1.79 (0.067)	9.11 (0.236)	1.91 (0.034)	1.62 (0.034)	5.01 (0.164)
ST/RH	WATS	1.63 (0.037)	7.68 (0.150)	2.49 (0.061)	1.91 (0.040)	4.77 (0.122)
ST/RH	HRIM	1.76 (0.097)	8.10 (0.252)	1.90 (0.065)	1.78 (0.061)	5.03 (0.218)
CLMI	WATM	4.05 (0.107)	17.71 (0.248)	4.04 (0.103)	2.91 (0.102)	12.49 (0.212)

Species	Population	Petiole length	Leaf length	Leaf width	Pollen color	Petal speckling	Plant height
CLST	CALF	14.95 (0.928)	48.05 (2.107)	11.80 (0.675)	3 (0)	2 (0)	4.65 (0.189)
CLST	MDWV	13.80 (0.907)	44.70 (2.608)	13.80 (0.694)	1 (0)	1.85 (0.082)	4.18 (0.347)
CLST	WILL	10.80 (0.462)	47.50 (1.946)	13.20 (0.866)	3 (0)	1.30 (0.105)	2.60 (0.156)
CLST	CONE	10.72 (1.084)	40.24 (2.820)	12.47 (0.589)	1.94 (0.250)	2.00 (0)	2.47 (0.266)
CLST	HUMB	8.15 (0.701)	40.80 (2.106)	13.65 (0.617)	1 (0)	1.60 (0.112)	1.59 (0.072)
CLST	MONT	12.45 (0.822)	46.35 (1.936)	13.10 (0.475)	2.1 (0.228)	1 (0)	2.78 (0.216)
CLRH	JCTH	16.45 (1.16)	57.05 (3.237)	15.90 (0.994)	5 (0)	3.76 (0.123)	5.93 (0.532)
CLRH	GANS	14.12 (1.150)	49.00 (2.650)	15.35 (0.813)	4.53 (0.212)	3.75 (0.106)	5.28 (0.392)
ST/RH	WATS	21.60 (0.933)	69.25 (2.076)	19.55 (0.709)	3 (0)	1.50 (0.199)	6.20 (0.260)
ST/RH	HRIM	7.69 (0.583)	31.88 (1.560)	9.88 (0.554)	3 (0)	2.13 (0.155)	1.91 (0.131)
CLMI	WATM	11.30 (0.821)	50.10 (2.376)	17.45 (1.150)	5 (0)	5 (0)	3.25 (0.160)

TABLE 4. MULTIPLE LINEAR REGRESSION FOR ALL STUDY POPULATIONS. N = 210, R = 0.828, standard error of estimate = 0.659. Dependent variable = petal speckling.

	Coefficient	SE	P		
Total petal length	0.298	0.080	<0.001		
Claw width	−0.598	0.214	0.006		
Isthmus width	0.656	0.298	0.029		
Leaf length	−0.034	0.012	0.006		
Pollen color	0.208	0.051	<0.001		
Plant height	0.215	0.043	<0.001		
Analysis of Variance					
	df	SS	MS	F	P
Regression	10	262.96	26.30	60.600	<0.001
Residuals	199	86.35	0.43		
Total	209	349.31	1.67		

width, claw length, and isthmus width (Appendix 1); between *C. stellata* and *C. mildrediae* for all characters except petiole length, leaf length, and plant height; and between *C. rhomobidea* and *C. mildrediae* for all characters except leaf length, leaf width, pollen color, and petal speckling. Populations that were ambiguously identified as "*C. stellata/C. rhomboidea*" showed significant differences with *C. stellata* for limb width and total petal length; with *C. rhomboidea* for limb width, total petal length, pollen color, petal speckling, and plant height; and with *C. mildrediae* for limb width, total petal length, claw width, claw length, isthmus width, pollen color, and petal speckling (Appendix 1). Uneven sample numbers, unequal variances, and non-normal data prevented an analysis of population-by-population differences.

A multiple linear regression with petal speckling as the dependent variable indicates there is a strong correlation with this trait and the independent variables, total petal length, claw width, isthmus width, pollen color, leaf length, and plant height (Table 4).

Genetic Analysis

Two primer combinations in the AFLP process yielded a total of 136 AFLP loci among 107 individuals. Mean total heterozygosity across all populations was 0.154 and ranged from 0.103 (GANS/CLRH) to 0.185 (JCTH/CLRH) within populations (Table 5).

TABLE 5. WITHIN POPULATION STATISTICS CALCULATED USING GENETIC DATA ANALYSIS (LEWIS AND ZAYKIN, 2002). P = polymorphic loci. He = Expected heterozygosity.

Population	n	P	He
MONT/CLST	15	0.577	0.166
CALF/CLST	6	0.342	0.123
WILL/CLST	16	0.592	0.194
CONE/CLST	14	0.612	0.158
WATS/STRH	19	0.622	0.181
HRIM/STRH	6	0.362	0.135
JCTH/CLRH	6	0.464	0.185
GANS/CLRH	11	0.398	0.103
WATM/CLMI	14	0.571	0.142
Mean	11	0.50	0.154

An analysis of molecular variance (AMOVA) shows that although there was genetic variation among the populations (13.19%), the majority of variation is found within populations (86.81%). Genetic differentiation among all populations was also low as calculated by Genetic Data Analysis (Φ_{st} = 0.132). Variance within populations was high 32.645 (sigma-G) and variance between populations (sigma-P) was 4.96.

Based on the 136 polymorphic loci across these nine populations, the Hickory analysis revealed similar $f = 0$ and full model DIC values of 3645.59 and 3642.35, respectively and provide weak evidence for inbreeding. Comparatively, a DIC value of 3665.0 from the $\theta = 0$ model indicates there is evidence for some differentiation among populations. The *f*-free model in Hickory gave a θ^B = 0.0137 (the Bayesian analog of G_{ST}) based on a mean *f* value of 0.5025 and a 95% credible interval of 0.2906 and 0.9811. The $\theta^B = 0.0137$ value is lower than traditional estimates of F_{ST} or G_{ST} because they assume total inbreeding or total outbreeding.

Distance matrix calculations and the corresponding neighbor joining tree indicate that populations consistently most closely related are: JCTH/r, MONT/s, CALF/s; CONE/s, WATM/m, GANS/r; and WILL/s, WATS/sr, HRIM/sr (Table 6, Fig. 1). A Neighbor-Joining phylogram was consistent with the AMOVA, Φ_{ST}, and Hickory data; there was no statistical significant clustering of any of the populations by initial species categorization (Fig. 1).

DISCUSSION

The purpose of this study was to determine whether there are a suite of characteristics that could be used to identify *C. stellata* from the sympatric species, *C. rhomboidea* and *C. mildrediae*, and if these characters were associated with measurable molecular variation. We have demonstrated

TABLE 6. DISTANCE MATRIX FOR NINE POPULATIONS BASED ON 136 AFLP LOCI USING PAUP 4b8 (SWOFFORD 2001). Nei (1978) identify above diagonal, coancestry distance below diagonal. See Table 1 for population labels. s = *stellata*, r = *rhomboidea*, sr = "*stellata/rhomboidea*", m = *mildrediae*.

Distance matrix	JCTH/r	MONT/s	CALF/s	WILL/s	WATS/sr	HRIM/sr	CONE/s	GANS/r	WATM/m
JCTH/r		0.978	0.962	0.936	0.921	0.944	0.948	0.951	0.957
MONT/s	0.041		0.973	0.952	0.943	0.964	0.962	0.967	0.971
CALF/s	0.098	0.072		0.960	0.945	0.965	0.971	0.976	0.974
WILL/s	0.181	0.163	0.111		0.981	0.977	0.955	0.954	0.959
WATS/sr	0.250	0.208	0.185	0.051		0.971	0.945	0.948	0.951
HRIM/sr	0.163	0.115	0.115	0.035	0.072		0.956	0.964	0.964
CONE/s	0.171	0.141	0.088	0.156	0.205	0.157		0.986	0.988
GANS/r	0.221	0.145	0.108	0.186	0.221	0.183	0.045		0.989
WATM/m	0.153	0.113	0.086	0.151	0.191	0.136	0.029	0.034	

that populations of *C. stellata* can be separated from *C. rhomboidea* based on all vegetative characteristics measured and all floral characteristics except claw width, claw length, and isthmus width. *Clarkia mildrediae* is easily differentiated from *C. stellata* for every character except leaf length, petiole length, and plant height; *C. mildrediae* differs from *C. rhomboidea* for all characters except petal speckling, pollen color, leaf width, and leaf length. Populations that were initially difficult to categorize as either *C. stellata* or *C. rhomboidea* were most similar to *C. stellata*; however, we were not able to identify a suite of characters that would distinguish these populations as either *C. stellata* or *C. rhomboidea*. Petal speckling can be predicted from a linear combination of the independent variables, total petal length, claw width, isthmus width, pollen color, leaf length, and plant height and these correlated characters may be interpreted to be a good suite of traits with which to identify species. This correlation, however, could simply mean that these characters are genetically linked regardless of species identification. Common garden experiments are needed for these taxa for further clarification on the inheritance and variability of these traits.

The analyses of molecular data indicate that most of the variation in the populations sampled is distributed across all populations. Species categorization is not consistent with the genetic data, and thus a conclusive determination about the evolutionary relationships among these populations cannot be determined here. Our data are consistent with genetic variation found in *Clarkia australis* and *C. virgata* with allozymes (Gottlieb and Ford 1999) and the high levels of allozyme variability found in *Clarkia dudleyana* that is not correlated with morphology (Podolsky 2001).

The distribution of molecular variation we found among these populations reflects shared variation between the study populations and is consistent

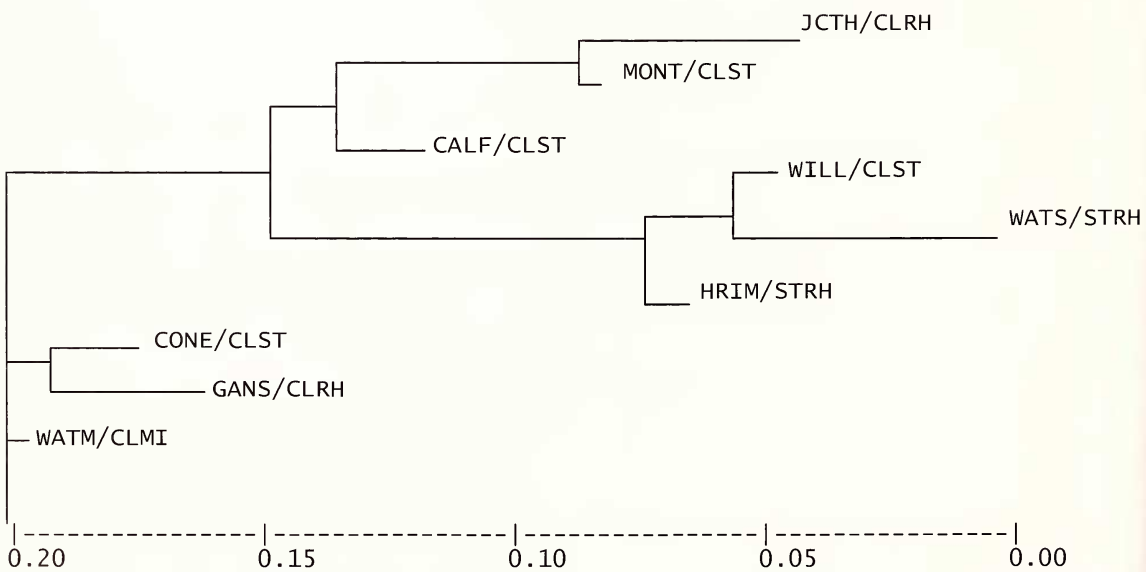

FIG. 1. Neighbor-joining tree for *C. stellata*, *C. rhomboidea*, and *C.* "*stellata/rhomboidea*", *C. mildrediae* populations showing genetic distances as measured by Saitou and Nei (1987). Species and location labels are referenced in Table 2.

with the derivation of *Clarkia stellata* from *C. mildrediae*, and *C. rhomboidea* from *C. mildrediae* and *C. virgata* (Gottlieb and Janeway 1997). We cannot conclude from these data that these populations represent different species. However, AFLPs are highly variable markers (Garcia-Mas et al. 2000), and additional techniques may provide a more precise estimate of relatedness among these populations. Although the possibility exists that same-sized AFLP fragments are not homologous across species, the close relatedness and recent evolution of these species should make a lack of homology unlikely. Additionally useful tools include restriction site analysis of the inter-transcribed spacer region of rDNA (ITS) or of non-coding regions of the chloroplast genome (cpDNA). Karyotype analysis within and among populations also may clarify the evolutionary relationships among these taxa. It is imperative if either ITS or cpDNA data are collected that a number of individuals are collected from each population. Rapid evolution within and hybridization among *Clarkia* species may otherwise obscure important differences in ambiguously identified populations.

We suggest that future genetic studies include more species and populations within *Clarkia* Section *Myxocarpa*. Field identification however, may never be simplified within *Clarkia* section *Myxocarpa*. A number of studies of this section have identified taxonomic difficulties due to recent speciation, local adaptation, rapid chromosomal evolution, sympatry, and hybridization (Mosquin 1966; Small 1971a, b; Grant 1981; Gottlieb 1995; Gottlieb and Ford 1999).

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Appendix 1. Kruskal-Wallis One Way Analysis of Variance on Ranks and all Pairwise Multiple Comparison Procedure (Dunn's Method) for *Clarkia stellata* (CLST), *C. rhomboidea* (CLRH), *C. stellata/rhomboidea* (STRH), and *C. mildrediae* (CLMI) Populations. Total petal length data were normally distributed ($P > 0.200$) with equal variances ($P = 0.012$) and were tested using a standard ANOVA.

Total petal length

Group	N	Mean	SD	SE
CLST	100	7.054	0.785	0.0785
STRH	53	7.785	0.890	0.122
CLRH	37	9.786	1.307	0.215
CLMI	20	17.705	1.111	0.248

Source of variation	df	SS	MS	F	P
Between species	3	1980.54	660.18	726.37	<0.001
Residual	206	187.23	0.91		
Total	209	2167.77			

All pairwise multiple comparison procedures (Tukey Test)

	Diff. of means	P value
CLMI vs. CLST	10.651	<0.001
CLMI vs. STRH	9.920	<0.001
CLMI vs. CLRH	7.919	<0.001
CLRH vs. CLST	2.732	<0.001
CLRH vs. STRH	2.002	<0.001
STRH vs. CLST	0.731	<0.001

Claw width

Species	N	Median	25%	75%
CLST	100	2.00	1.900	2.200
STRH	53	2.00	1.600	2.425
CLRH	37	2.00	1.900	2.400
CLMI	20	3.95	3.750	4.300

$H = 55.362$, df = 3, $P = < 0.001$

Comparison	Diff. of ranks	Q value	$P < 0.05$
CLMI vs. STRH	108.340	6.794	Yes
CLMI vs. CLST	106.320	7.143	Yes
CLMI vs. CLRH	96.649	5.731	Yes
CLRH vs. STRH	11.691	0.898	No
CLRH vs. CLST	9.671	0.827	No
CLST vs. STRH	2.020	0.196	No

Claw length

Species	N	Median	25%	75%
CLST	100	1.500	1.300	1.600
STRH	53	1.600	1.500	1.825
CLRH	37	2.200	1.700	2.525
CLMI	20	4.000	3.650	4.400

$H = 108.708$, df = 3, $P = < 0.001$.

Comparison	Diff. of ranks	Q value	$P < 0.05$
CLMI vs. STRH	131.060	8.805	Yes
CLMI vs. CLST	96.519	6.053	Yes
CLMI vs. CLRH	96.649	2.770	Yes
CLRH vs. STRH	11.691	7.213	No
CLRH vs. CLST	9.671	3.826	No
CLST vs. STRH	2.020	3.346	No

Appendix 1. Continued.

Isthmus width

Species	N	Median	25%	75%
CLST	100	1.700	1.600	1.800
STRH	53	1.800	1.500	1.925
CLRH	37	1.800	1.600	2.000
CLMI	20	2.900	2.600	3.100

$H = 63.122$, df = 3, $P < 0.001$.

Comparison	Diff. of ranks	Q value	$P < 0.05$
CLMI vs. CLST	116.395	7.820	Yes
CLMI vs. STRH	95.520	5.990	Yes
CLMI vs. CLRH	86.931	5.155	Yes
CLRH vs. CLST	29.464	2.520	No
CLRH vs. STRH	8.589	0.660	No
STRH vs. CLST	20.875	2.002	No

Limb width

Species	N	Median	25%	75%
CLST	100	4.100	3.700	4.500
STRH	53	4.700	4.100	5.000
CLRH	37	5.200	4.675	5.725
CLMI	20	12.650	11.900	12.950

$H = 98.685$, df = 3, $P < 0.001$.

Comparison	Diff. of ranks	Q value	$P < 0.05$
CLMI vs. CLST	130.375	8.759	Yes
CLMI vs. STRH	91.660	5.748	Yes
CLMI vs. CLRH	55.527	3.292	Yes
CLRH vs. CLST	74.848	6.401	Yes
CLRH vs. STRH	36.133	2.776	Yes
STRH vs. CLST	38.715	3.750	Yes

Petiole length

Species	N	Median	25%	75%
CLST	100	11.000	9.000	15.000
STRH	53	11.000	8.000	19.000
CLRH	37	16.000	11.000	19.000
CLMI	20	11.000	8.000	13.500

$H = 11.672$, df = 3, $P = 0.009$.

Comparison	Diff. of ranks	Q value	$P < 0.05$
CLRH vs. CLMI	45.080	2.673	Yes
CLRH vs. CLST	36.605	3.131	Yes
CLRH vs. STRH	24.490	1.881	No
STRH vs. CLMI	20.590	1.291	No
STRH vs. CLST	12.115	1.173	No
CLST vs. CLMI	8.475	0.569	No

Leaf length

Species	N	Median	25%	75%
CLST	100	44.000	38.500	52.000
STRH	53	43.000	32.750	64.250
CLRH	37	53.000	44.000	63.500
CLMI	20	48.000	42.500	57.500

$H = 8.949$, df = 3, $P = 0.030$.

Appendix 1. Continued.

Comparison	Diff. of ranks	Q value	P < 0.05
CLRH vs. CLST	32.970	2.820	Yes
CLRH vs. STRH	25.531	1.961	No
CLRH vs. CLMI	10.410	0.617	No
CLMI vs. CLST	22.560	1.516	No
CLMI vs. STRH	15.121	0.948	No
STRH vs. CLST	7.439	0.720	No

Leaf width

Species	N	Median	25%	75%
CLST	100	13.000	11.000	15.000
STRH	53	14.000	10.750	18.000
CLRH	37	15.000	13.000	18.250
CLMI	20	16.500	14.500	20.000

H = 20.331, df = 3, P < 0.001.

Comparison	Diff. of ranks	Q value	P < 0.05
CLMI vs. CLST	5.315	3.716	Yes
CLMI vs. STRH	41.344	2.593	No
CLMI vs. CLRH	17.311	1.026	No
CLRH vs. CLST	38.004	3.250	Yes
CLRH vs. STRH	24.032	1.846	No
STRH vs. CLST	13.971	1.353	No

Pollen color

Species	N	Median	25%	75%
CLST	100	3.000	1.000	3.000
STRH	53	3.000	3.000	3.000
CLRH	37	5.000	5.000	5.000
CLMI	20	5.000	5.000	5.000

H = 139.364, df = 3, p < 0.001.

All pairwise multiple comparison procedures (Dunn's Method)

	Diff. of ranks	Q value	P < 0.05
CLMI vs. CLST	114.465	7.690	Yes
CLMI vs. STRH	89.330	5.602	Yes
CLMI vs. CLRH	68.216	0.487	No
CLRH vs. CLST	106.249	9.087	Yes
CLRH vs. STRH	81.114	6.231	Yes
STRH vs. CLST	25.135	2.434	No

Petal speckling

Species	N	Median	25%	75%
CLST	100	2.000	1.000	2.000
STRH	53	2.000	2.000	2.000
CLRH	37	4.000	3.000	4.000
CLMI	20	5.000	5.000	5.000

H = 148.086, df = 3, P < 0.001.

Comparison	Diff. of ranks	Q value	P < 0.05
CLMI vs. CLST	131.400	8.828	Yes
CLMI vs. STRH	106.774	6.696	Yes
CLMI vs. CLRH	28.270	1.676	No
CLRH vs. CLST	103.130	8.820	Yes
CLRH vs. STRH	78.503	6.030	Yes
STRH vs. CLST	24.626	2.385	No

Appendix 1. Continued.

Plant height

Species	N	Median	25%	75%
CLST	100	3.000	2.000	4.250
STRH	53	2.500	2.000	5.625
CLRH	37	5.500	4.000	7.000
CLMI	20	3.000	3.000	3.500

H = 36.560, df = 3, P < 0.001.

Comparison	Diff. of ranks	Q value	P < 0.05
CLRH vs. CLST	69.606	5.953	Yes
CLRH vs. STRH	58.682	4.508	Yes
CLRH vs. CLMI	58.641	3.477	Yes
CLMI vs. CLST	10.965	0.737	No
CLMI vs. STRH	0.042	0.003	No
STRH vs. CLST	10.923	1.058	No